Supplementary information

Methods:

Genomic DNA was extracted from each of five core sections using a FastDNA SPIN kit (for Soil) (Qbiogene, Carlsbad, CA). For each section, five separate extractions were performed and DNA from each extraction was pooled to minimize non-systematic extraction and sampling biases. PCR to obtain DNA from *Bacteria* for DGGE was performed by amplifying partial 16S rRNA genes using primer 341F with a 5'-end 40bp GC clamp (collectively designated as 341F-GC) and primer 518R (referred to as primer 3 and primer 2, respectively, by Muyzer et al (10). PCR conditions were as described by Kulp and colleagues (6) with the following modifications: KCl concentration was 50 mM, $MgCl_2$ concentration was 2 mM, and 200 μM primers were used for 27F/1492R and M13F/M13R amplifications. Nested PCR was used to obtain a bacterial PCR product from sample 5III as described previously (6). Nested PCR was also required to obtain DNA for archaeal DGGE PCR. The 16S rRNA gene primers Arch21F and Arch958R (3) were used for first round amplification with genomic DNA as template. Then 1μl of the PCR product was used as template for a second round 16S archaeal DGGE PCR using primers PARCH340f with a GC clamp (collectively designated as PARCH340f-GC) and PARCH519r (11). 5III section yielded no archaeal DGGE PCR product.

Eluted DNA was used as template for re-amplification using primers lacking the GC-clamp (6), the re-amplified PCR product was sequenced with primer 341F (*Bacteria*) or primer PARCH340f (*Archaea*) and sequences were analyzed as previously described (6).

For clone library construction, universal primers for *Bacteria* 27F (7) and 1492R (2) were used to amplify the nearly full-length 16S rRNA gene from genomic DNA. The PCR product was purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Purified PCR product was cloned into a pCR Topo 2.1 vector and transformed into TOPO competent DH5α cells (Invitrogen, Carlsbad, CA), according to manufacturer's instructions. White colonies were picked and checked for the presence of inserts by PCR amplification with vector primers M13F and M13R. PCR products of the expected size were digested separately with restriction enzymes *Hha*I and *Msp*I (New England Biolabs, Ipswich, MA) for Restriction Fragment Length Polymorphism (RFLP) analysis as previously described (8).

To confirm the RFLP results, partial sequencing of the 5' end of the 16S rRNA gene was obtained using the universal primer 907R (14). At least one clone from each putative OTU was sequenced. All of the 907R sequences were aligned in ClustalW (version 1.83 for Windows XP) under the settings of a gap-opening penalty of 10.0 and a gap-extension penalty of 0.1 for pairwise and multiple alignments then the alignment file was used to calculate a distance matrix in Phylip (version 3.65). The resulting distance matrix by a Jukes-Cantor model was used as the input for DOTUR adopting the furthest neighbor cluster algorithm with 3% distance difference used as cutoff for grouping into OTUs (12). In

most cases, DOTUR assigned clones to the same OTU as the RFLP; in case of a conflict in the assignment, sequence-based DOTUR was considered the definitive method. All sequences in this study were inspected for chimeras using Pintail (1) and the Chimera_Check program in the Ribosomal Database Project website (http://rdp.cme.msu.edu/). Chimeric sequences confirmed by both methods were discarded from analyses.

Sequences of highly represented OTUs (those with five or more clones) generated from the 907R sequencing primer were aligned using Webaligner (SILVA, http://www.arb-silva.de/aligner/). Alignments were imported into ARB (9) and checked manually in ARB_EDIT (www.arb-home.de); only unambiguous positions were included in phylogenetic analyses. Phylogenetic trees were constructed in ARB by a maximum-likelihood method through the PHYML (DNA) implementation. Default parameters were used and 100 bootstrap replicates were performed. Since clones from the same OTU (at least two sequenced clones for each of these major OTUs) showed negligible divergence in phylogenetic trees, only one representative clone from each OTU is displayed in the trees; the number of clones belonging to that OTU are shown. Distance matrices and neighbor-joining trees were generated by Phylip using a Jukes-Cantor model from ClustalW alignments were used as input files for $\int$-LIBSHUFF (12) and TreeClimber (13), respectively. The Shannon diversity index (5), Good's coverage (4), and Chao-1 estimator of richness (5) were calculated for each sample's clone library and their combined libraries on the basis of the OTU distribution (Table 1).

Supplemental table 1: PCR conditions used in this study

Pimer Pair		PCR conditions											Reference
		Initial Denaturation		No. of cycles	Denaturation		Annealing		Elongation		Further Extension		
		Temp(°C)	Time(s)		Temp(°C)	Time(s)	Temp(°C)	Time(s)	Temp(°C)	Time(s)	Temp(°C)	Time(s)	
Bacteria	341F-GC/518R for DGGE	94	300	20/10	94	30	65/55a	30	72	30	72	420	Kulp et al.
	341F/518R	94	300	30	94	30	55	30	72	30	72	420	This study
	27F/1492R	94	300	30	94	60	50	60	72	60	72	420	This study
Archaea	Arch21F/Arch958R	94	120	35	94	30	50	30	72	45	72	300	This study
	PARCH340f -GC/ PARCH519r for DGGE	95	300	30	95	60	53.5	30	72	60	72	420	This study
	PARCH340f /PARCH519r												This study
Vector	M13F/M13R	94	180	30	94	30	55	30	72	45	72	300	This study

[a] The program consisted of a touchdown protocol where the initial annealing temperature decreased by 0.5°C each cycle during the first 20 cycles.

Supplemental table 2: BLAST results of Archaea DGGE sequences

Archaea DGGE band	Representative nearest neighbor by BLAST				
	Definition	**Accession No.**	**Similarities**	**Phylum**	**Isolation Source**
Archaea-1	Uncultured Candidatus Nitrosopumilus sp. clone 9	GU386315	97/97 (100%)	*Thaumarchaeota*	Pelagic central Baltic Sea redoxcline
Archaea-2	Uncultured Nitrosopumilaceae archaeon clone GG101008Arch21	JN592005	97/105 (92%)	*Thaumarchaeota*	Surface seawater, Puget Sound
Archaea-3	Uncultured Candidatus Nitrosopumilus sp. clone 9	GU386315	95/95 (100%)	*Thaumarchaeota*	Pelagic central Baltic Sea redoxcline
Archaea-5	Uncultured archaeon clone AS17-35	AF225693	98/101 (97%)	*Crenarchaeota*	Rice field soil
Archaea-6	Uncultured archaeon clone 5E_07B	JX099326	94/97 (97%)	*Thaumarchaeota*	> 6000 meters elevation mineral soils of Atacama desert
Archaea-7	Uncultured archaeon clone gls_13	AB583874	92/96 (96%)	*Crenarchaeota*	Upland field soil
Archaea-8	Uncultured crenarchaeote clone F160cmFL252	JN002691	95/95 (100%)	*Crenarchaeota*	Serpentinized dunite

SUPPLEMENTAL TABLE 3: *p* – Values for TreeClimber and ∫-LIBSHUFF analyses

Analysis	**4I vs. 4II**	**4I vs. 5I**	**4II vs. 5I**
TreeClimber	0.028	0.055	0.021
∫-LIBSHUFF*	0.0077; 0.81	0.034; 0.98	0.010; 0.24

*Values are from the two ∫-LIBSHUFF tests of the former sample versus the latter sample, and vice versa